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(Article begins on next page)

Surgical sterilization of male and female grey squirrels (*Sciurus carolinensis*) of an urban population introduced in Italy

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Running head: SURGICAL STERILIZATION OF GREY SQUIRRELS

ABSTRACT

We report a successful surgical sterilization procedure for population control of 324 male and female free-ranging grey squirrels (*Sciurus carolinensis*) in Genoa (Italy). We describe the clinical procedure from the trapping of the animals to their surgical sterilization and release in another part of the city. Live-trapped squirrels were transported to the veterinary clinic within 1–2 hr of capture and maintained in a hospitalization room reserved for them. The waiting period before surgery was kept below 12 hr. The developed procedure has resulted in a survival of 94% of trapped squirrels from surgery to animal release. Sterilized squirrels started to feed in a very short time (1.0–1.5 hr), and after 2–3 days, it was possible to release them in a new area. Amoxicillin was used as a long-acting postoperative antibiotic to reduce the period of captivity. The successful surgical procedure described here can provide an important additional tool for the management of introduced populations of squirrels. We showed that the surgical sterilization of some hundred squirrels is clinically possible and could be included in management strategies aimed at removing critical populations of these species. Moreover, the data allow dosages and operational times in order to provide economic viability assessment of future population control measures.

KEY WORDS: castration, eradication, ovariectomy, invasive species, population control, species management, surgery.

INTRODUCTION

The introduction of non-native species is nowadays considered one of the leading causes of progressive loss of biodiversity [1] and imposes important costs on society [18]. Therefore, eradication projects are becoming more and more common with important benefits for biodiversity conservation [15].

The grey squirrel (*Sciurus carolinensis*) is an American species introduced repeatedly in Europe in the 19th and 20th centuries in Great Britain, Ireland and Italy [3]. In Italy, many separate introductions took place in different regions in the northern and central parts of the country [16,17,21]. The species is considered invasive for its ability to adapt to European habitats and its competition with the native Eurasian red squirrel (*Sciurus vulgaris*) [2,6]. The competition is based on a better use of food resources by the grey squirrel [26,27]. In Great Britain, it is also disease-mediated, as the introduced species acts as a reservoir host for the squirrel poxvirus, which causes high mortality in red squirrels [24]; in Italy the virus is not reported [20]. The Italian grey squirrel populations are spreading, threatening the red squirrel at a large scale [7,10,11].

The grey squirrel is actively managed in Italy to reduce its spread and avoid the local extinction of the red squirrel [8,23]. The species is generally controlled through live trapping and euthanasia [6,7]. However, to eradicate an urban population localized in a green area inside the city of Genoa, we planned a project involving capturing, surgically sterilizing and releasing the animals in another green area of comparable size of the same city. This decision was made because squirrels have a strong emotional impact on local people and had become a popular attraction in the park. Surgical sterilization was chosen to make the removal of the animals acceptable to most citizens and NGOs [4,12]. For the release of the sterilized animals, an urban park 6 km away from the trapping area was selected, after a survey of all the parks inside the city, taking into consideration the suitability for squirrels (tree cover, food availability), the isolation from areas where the native Eurasian red squirrel was present and from the catch area. The intervention was considered feasible because the squirrel population was estimated at 200–300 animals confined to a small urban area of about 12 ha [25], and economic resources were available from a European LIFE project.

The aim of this paper is to report the clinical procedure developed to sterilize free-ranging grey squirrels in Genoa, from the trapping of the animals to their surgical sterilization and release.

MATERIALS AND METHODS

Ethics statement

All procedures complied with Italian laws. The management plan that oversaw trapping and handling of the squirrels was subject to advice from the Institute for Environmental Protection and Research (ISPRA), and permission for trapping and removing squirrels was given by the wildlife service of Genoa Province. Grey squirrels were removed and sterilized within a European project (LIFE09 NAT/IT/000095 EC-SQUARE) with the aim of implementing management action on the grey squirrel in northern Italy for the conservation of the native Eurasian red squirrel. Procedures followed the indications reported in the EU Directive 2010/63/EU.

Animals

From January 2014 to March 2015, in about 130 trapping sessions corresponding to 1,654 trap-days, 324 squirrels were trapped using 10–26 Tomahawk Live Traps Collapsible Model 202 (Hazelhurst, WI, U.S.A.) baited with hazelnuts and walnuts. In the first phase, when population density was high, groups of traps were continuously kept under visual inspection by operators. In the second phase, traps were visually inspected every 10–15 min. Trapped squirrels were maintained inside the traps and transported to the veterinary clinic within 1–2 hr of capture.

Surgical procedures

In the clinic, squirrels were admitted to a hospitalization room reserved for them, in which any source of stress (light, olfactory and auditory) was minimized. The waiting period before surgery was kept below 12 hr (range: 1–12 hr).

To induce surgical anaesthesia, squirrels were intramuscularly administered a combination of 0.02 mg/kg dexmedetomidine (Dexdomitor® Elanco, Sesto Fiorentino, FI, Italy) and 15.0–20.0 mg/kg ketamine (Lobotor® Acme, Corte Tegge – Cavriago, RE, Italy) [9]. After 5–10 min, the immobilized squirrels were transferred to the surgical table. Here, they received isoflurane (Isovet® Piramal Healthcare, Morpeth, UK) in oxygen (0.5 l/min) via face mask; concentration was maintained at 1.5%. As integration to the analgesic protocol, 0.3 mg/kg of meloxicam (Metacam® Ceva, Libourne, France) was administered subcutaneously. Monitoring of vital signs (i.e. heart rate, blood pressure, etc.) was not available during surgery.

The animals were then clinically examined and biological information (sex, weight and hind foot length) was collected. Blood was drawn from the jugular vein and faecal samples were also taken

from the rectum for a broad surveillance plan on infectious diseases [19]. The squirrels were then placed in dorsal recumbency and an area of approximately 3.0×5.0 cm in the caudal abdominal region was clipped and aseptically prepared using a 4.0% chlorhexidine (Clorexym® ICF, Scannabue, CR, Italy) solution.

In adult males, a single 2.5–3.0 cm-long, midline, prescrotal skin incision was made using a No. 15 scalpel blade. The underlying subcutis was sharply incised, enabling the externalization of a testicle. An opening was then made in the parietal vaginal tunic, and we proceeded to lace the artery lacing, the testicular vein and the spermatic cord with monofilament absorbable suture 4-0 (Biosyn® Covidien, Minneapolis, MN, U.S.A.). The testicle was then removed by severing the vessels and umbilical cord with blunt scissors. The same procedure was conducted on the contralateral testicle. Subsequently, the surgical breach was closed with a suture of the vaginal tunica and of the skin, using a 4-0 synthetic polyester absorbable monofilament suture (Biosyn® Covidien, Minneapolis, MN, U.S.A.).

Young, non-reproductive males had smaller testicles (<1 cm), localized in the subcutaneous position; the scrotum was hardly visible. In these animals, a gentle compression of the abdomen in the cranio-caudal direction facilitated the descent of the testicles in the scrotum. The surgery was then carried out by the same procedure as for the adults, with the skin incision limited to 2 cm.

In females, the skin incision in the midline of the abdomen was approximately 2.0 cm long. The subcutis was separated, and then the abdominal wall was scored with a blade and enlarged with blunt scissors. The ovaries (approximately 0.3 cm) are localized at the level of the lumbar portion of the abdomen. Using a small hook, the uterine horns were engaged and exteriorized. This allowed the visualization of the ovaries. The vein and the ovarian artery were then fastened with 4-0 absorbable monofilament suture (Biosyn® Covidien, Minneapolis, MN, U.S.A.); finally, the ovaries were removed by cutting the ovarian pedicle with blunt scissors. When it was necessary to remove the uterus (i.e. pregnancy), a suture was made at the level of the uterine cervix. The surgery was completed with a continuous suture of the abdominal wall and skin with monofilament absorbable 4-0 (Biosyn® Covidien, Minneapolis, MN, U.S.A.).

All squirrels received 15 mg/kg long-acting Amoxicillin (Solmox® Zoetis, Parsippany, NJ, U.S.A.) subcutaneously as the postoperative antibiotic. Finally, a Pit-Tag (Indexel® Merial Boehringer Ingelheim, Ingelheim am Rhei, Germany or Therachip® Bioforlife, Lugano, Switzerland) was inserted in the interscapular subcutaneous location for individual recognition. After the surgical operation, each squirrel was placed in a wire-mesh cage (60x30x40 cm) in a

post operating room and baited with a mixture of nuts, hazelnuts, peanuts and small portions of apple, with water *ad libitum*. Squirrels were monitored closely for 6–8 hr for postoperative follow-up and released once they showed spontaneous feeding and good vitality.

RESULTS

In total, we sterilized 321 squirrels: 272 adults (116 females and 156 males) and 49 juveniles (27 females and 22 males). Other three animals died after capture but before surgery. Mean weight (\pm SD) was 404 ± 44 g for adult males, 402 ± 44 g for adult females, 279 ± 65 g for young males and 287 ± 92 for young females. The overall medical needs required for the sterilization of an individual grey squirrel are summarised in Table 1. Isoflurane 1.5% in oxygen, associated with meloxicam, provided excellent surgical-stage anaesthesia and allowed for safe surgical interventions. Animals were awake 15–20 min postoperatively, and they began to feed after 1.0–1.5 hr. The entire procedure, from induction of anaesthesia with dexmedetomidine and ketamine to animal awakening lasted 1.0–1.5 hr. Anesthesia lasted approximately 35 minutes and operation time was about 15 minutes. The postoperative hospital stay was 2–3 days (females: 3.1 ± 1.2 ; males 2.7 ± 0.8). Afterwards, the animals were released in another park in the city of Genoa where nest boxes and feeding stations were provided and checked regularly.

In the 2–3 days of postoperative period from surgery to animal release, 20 animals (6.2% of sterilized animals) died; 6 were males (3.4% of males) and 14 females (9.8% of females). These animals showed signs ranging from exhaustion to hypothermia, while wounds did not show any complication such as dehiscence or sign of infection.

The autopsy of the animals that died before and after sterilization showed that 10 of these (3.1% of the total; 3 died before and 7 after sterilization) were in poor physical condition before capture, while the others (13 animals, 4.0 % of the total) showed aspecific signs of lung and heart impairment (congestion, emphysema, dilation) not associated to particular complications during surgery and anaesthetic intervention. Other four animals captured in poor physical conditions were treated, sterilized and released in better conditions. Faecal samples were positive for oocysts in 31 out of 40 (77.5%) animals examined, while two animals were positive for chigger mite *Neotrombicula talmiensis* [22]. We could not find any correlation between these parasites and the death of some animals.

Since the squirrels were released after the postoperative hospital stay, it was not possible to estimate the long-term survival rate. However, a first comparison between the squirrels released after sterilization in the new area and those left in the original park showed similar behaviours

(including the construction of nests), but differences in the hours of maximum activity, probably dependent on the different attendance of the public in the two parks [14]. Furthermore, 18 sterilized and marked animals were released in the original park in Genoa Nervi to evaluate the consistency of the remaining population through a mark and recapture procedure. Sixteen of the animals (89%) were recaptured after a time varying from 2 to 77 days (median: 25.5 days) in excellent condition, one (5.5%) was found road killed and only one animal has not been recaptured. Excluding the road-killed animal, we could therefore estimate the postoperative survival of the released animals to be $\geq 94.5\%$ within the first 2 months.

DISCUSSION

This is the first report on the successful surgical sterilization of a large number of male and female grey squirrels for population control. In 14 months, we captured and sterilized 324 squirrels. Afterwards, only few squirrels were observed and trapped in the area, indicating that most of the animals had been removed. Considering the females that would have reproduced before being captured, the initial estimation of approximately 200–300 animals is considered accurate.

Overall, postoperative survival was 93.8% in the three days before release and in a subsample of animals, the estimated survival after release was $\geq 94.5\%$ within two months. About half of the dead animals were in poor condition before trapping. For the others, the aspecific pathological findings did not allow us to ascertain the cause of death. Moreover, the absence of individual clinical history, which frequently follow medical intervention on wild animals, highlight the difficulties to operate in field conditions. In particular, since no association with surgical and anaesthetic complications was observed distress related to capture and transport or surgical and anaesthetic procedures could not be discarded. However, active traps were monitored continuously by field worker, which covered trapped animals with a cloth within 10–15 minutes after capture. Subsequently, animals were transported to the clinic within a few hours, keeping them in darkened cages. In the absence of physiological reference parameters and monitoring of vital signs to evaluate the animal condition and in absence of an anaesthetic protocol for squirrels, the procedure we adopted during sterilization was derived from protocols applied on small rodents [13], reducing anaesthetic concentration in order to prevent overdoses. For example, in absence of reference values for dosing meloxicam in squirrels, we chose the lowest value of the dosage interval to prevent overdosing. Indeed, even with lower doses the protocol applied provided a satisfying level of anaesthesia, constituting a simple and effective starting point to be

used, even in field conditions where surgical equipment is limited. However, we recognise the need to define a specific anaesthetic protocol and the improvement of drug association tailored on squirrel physiology, in order to further reduce any possible complication and subsequent mortalities.

It should also be considered that nearly half of the animals that died before surgery or in the postoperative period were in poor physical condition, suggesting a condition of malnutrition, probably connected to the high density of the squirrel population in the park at the beginning of the project (estimated density in 2011: 19.5 ind./ha, C.I. 95%: 13.1–29.1, A. Balduzzi and A. Marsan, unpublished data).

The procedure described in this study proved to be feasible and successful for the surgical sterilization of both male and female squirrels. With regard to the anatomy of the females, it should be emphasized that the median line of the abdomen, where the surgical incision must be made for sterilization, is poorly visible. It was possible to accurately locate the position using as landmarks the vertices of a triangle formed from the vulva and the abdominal nipples. The centre of this triangle turned out to be very close to the linea alba. This allowed us to avoid incision of the mammary blood vessels, which were poorly visible but very bloody.

Squirrels recovered their condition shortly after the surgery. They started to feed in a very short time, and after 2–3 days, it was possible to release them in the new urban park. In fact, the decision to administer long-acting Amoxicillin as a postoperative antibiotic was due to the decision to release the animals as soon as possible to reduce the stress of captivity. This is an important point when the animals to be sterilized are part of a relatively large population. The hospitalization of a large number of animals would be difficult and expensive to arrange, severely limiting the applicability of this method.

Squirrels are a group of animals commonly traded as pets and often introduced either through escapes from captivity or after deliberate releases of animals [3,16]. In many cases, squirrels are released in urban areas, establishing viable populations and attracting people [4,16]. In these cases, management intervention involving euthanasia may not be accepted, leading to a strong opposition to the entire conservation project. In such a situation, the surgical sterilisation of animals may be an alternative management option to reduce opposition and gain local support. The costs of this intervention can vary between countries and time (i.e. cost of manpower and equipment), but in general it is higher with more complex logistical organization in respect to live trapping and euthanasia. The feasibility of surgical sterilization and its economic sustainability should therefore be carefully assessed before considering this procedure a management option.

The successful surgical procedure described in this study and the high survival rate of the sterilized animals provides an important additional tool for the management of introduced populations of squirrels when strong opposition of the local community threaten the control program. We demonstrate that the surgical sterilization of some hundred squirrels is clinically feasible for small and isolated populations and could be included in management plans that foresee the removal of critical populations.

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Table 1. Medical supplies and operational times required for surgical sterilisation of grey squirrels.

Item	Dose	Individual dosage*	Time
<i>Medical used drug</i>			
Dexmedetomidine	0.02 mg/kg	0.01 mg	
Ketamine	15.0-20.0 mg/kg	7.5-10 mg	
Isoflurane	1.5%		
Meloxicam	0.3 mg/kg	0.15 mg	
4.0% chlorhexidine			
Amoxicillin	15 mg/kg	7.5 mg	
<i>Surgical material</i>			
Absorbable monofilament suture 4-0			
<i>Procedure times</i>			
Time for surgery			1-1.5 hr
Time for postoperative in hospital follow-up			2-3 days

* Individual dosage is estimated on an average body weight of 500 g.